Research Article

Effects of metformin on omentin levels in a newly diagnosed type II diabetes mellitus: Randomized, placebo controlled study

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Abstract

Metformin is one of the most common used anti-diabetes drugs for treatment of T2DM. Metformin is considered as anti-hyperglycemic drug, it lowers blood glucose levels in T2DM without producing significant hypoglycemia. Plasma omentin-1 concentrations and expression of its mRNA in human omental adipose tissue were significantly lower in patients with impaired glucose tolerance and T2DM. The objectives of the present study were to establishing and elucidation of omentin-1 serum levels in metformin treated T2DM patients. The selected subjects patients were divided into two groups: Group A: includes thirty of a newly diagnosed T2DM patients with (mean age 49.32± 11.18 years), were initially treatment with metformin at time diagnosis according to ADA criteria. Group B: includes thirty healthy volunteers. The duration of treatment was three consecutive months. After a period of 12 hrs fasting, the blood samples (10ml) were withdrawn from all subjects by vein puncture before starting the study (before starting metformin) and after three months of metformin treatment. There was a significant difference in the baseline serum omentin-1 levels between metformin and control groups (p=0.023) and significant rising in serum omentin-1 levels after three month s duration of treatment with metformin. Conclusions: In newly diagnosed T2DM patients, omentin-1 levels were lower compared to control subjects. Three months of treatment with metformin lead to in a significant elevation in omentin-1 serum levels compared with baseline values.

Key words: *Metformin, Omentin-1, T2DM*

INTRODUCTION

Type 2 diabetes mellitus represents 90 - 95% of the overall diabetes types worldwide [1]. The incidence of T2DM is increasing nowadays, primarily due to increases in the prevalence of consumption of high-calorie diets, obesity and sedentary lifestyle [2]. Obesity alone has been founded to be a contributing factor to around 55% of T2DM [3]. Most patients with T2DM exhibited abdominal obesity, which contributes for development of insulin resistance [4].

T2DM usually affects individuals who are obese and most of the cases are diagnosed at age more than forty years. However, the demographic profile of T2DM is changing nowadays, where the prevalence of T2DM is increasing among young adults and even children, pathological abnormalities in T2DM are impairment of insulin secretion from a dysfunctional pancreatic β-cell and/or insulin action due to development of insulin resistance [5]. Insulin resistance is the most important risk factor for T2DM and is characterized by inability of the target tissues to respond for insulin action [6]. In response, the pancreatic β-cells needed to secrete an extra amount of insulin for maintenance of euglycemic state, however; with time, the defects in the secretion of insulin will prevent the pancreatic β-cells from preserving a high rates of insulin secretion. Consequently, this will result in impairment of glucose tolerance and eventually development of T2DM [7]. Insulin resistance may be exists for several years before diagnosis of DM and continue to progress during the course of the disease [8].The chronic elevation in glucose and lipid levels will causes gluco-lipotoxicity, which in turn contributes to pancreatic β-cell failure via

activation of the stress response, enhanced apoptosis and exacerbates insulin resistance. Metformin is one of the most common used anti-diabetic drugs for treatment of T2DM [9]. Metformin is considered as anti-hyperglycemic drug, it lowers blood glucose levels in T2DM without significant hypoglycemia [10]. The glucose-lowering properties of metformin are inhibition of gluconeogenesis , glycogenolysis and enhancing insulin-stimulated glucose uptake by skeletal muscle and adipocytes [11].However, the main effect of metformin appears to be through decreasing hepatic glucose output due to inhibition of respiratory-chain complex 1 in the mitochondria, causing transient reduction in the status of cell energy which promotes stimulation of adenosine monophosphate -activated protein kinase (AMPK), that plays an important role in regulating of energy balance, moreover, within skeletal muscle, stimulation of AMPK increases glucose uptake and lipid oxidation while; in liver stimulation of AMPK decreases gluconeogenesis and synthesis of the lipids[12].

Omentin-1 was initially found in intestinal cell (called intelectin), it is mainly expressed at visceral adipocity and low levels of omentin expression have been establish in human muscle, kidney, intestine, endothelial cells, cardiac tissues placenta and ovary [13]. Abnormal omentin-1 secretion is thought to have a role in the pathophysiology of insulin resistance, inflammatory processes, endothelial dysfunction and cardiovascular diseases [14].Plasma omentin-1 concentrations and expression of its mRNA in human omental adipose tissue are significantly lower in patients with impaired glucose tolerance and T2DM [15]. In T2DM patients, fasting serum omentin-1 levels were found to be linked negatively with insulin level and HOMA-IR [16]. A newly diagnosed T2DM female patients had been found to have considerably low serum levels of omentin-1 than age-matched control females, also both diabetic and control groups with insulin resistant have appreciably lower serum omentin-1 levels than patients without insulin resistance, as insulin resistance worsened, omentin-1 levels will decline [17]. In addition, lower circulating omentin-1 levels are documented in T2DM patients [18].

Thus, the objectives of the present study were establishing and elucidation of omentin-1 serum levels in metformin treated T2DM patients.

PATIENTS AND METHODS

This study was carried out on thirty healthy control subjects (mean age 47.8± 9.3 years), and thirty T2DM patients, who attended the Endocrinology and Diabetes specialized center, Al-Mustansiriya University – Baghdad/Iraq. The control subjects did not have any medical disorders and were not receiving any medications. The enrolled patients attended the Endocrinology and Diabetes Center from December 2014 till June 2015. The selected subjects patients were divided into two groups:

Group A: includes thirty of a newly diagnosed T2DM patients with (mean age 49.32± 11.18 years), were initially treatment with metformin at time diagnosis according to ADA criteria.**Group B**: includes thirty healthy volunteers.The duration of treatment was three consecutive months.

The following exclusion criteria include patients with:

1- Type 1 diabetes
2-Chronic illnesses of heart, kidney, thyroid, lung or liver.
3-Corticosteroids therapy.
4-Hormonal replacement therapy
5-Acute renal failure.
6-Malignancy.

Anthropometric measurements: Blood pressure, height (cm), weight (kg) and body mass index BMI were determined for all patients at the initiation of the study and 3 months later. BMI=weight (kg)/height (cm) ^{2}[19].

Biochemical measurements: After a period of 12 hrs fasting, blood samples (10ml) were withdrawn from all subjects by vein puncture before starting the study (before starting metformin) and after three months of metformin treatment. 9 mls of blood were placed in plane tubes, and 1 ml was placed in EDTA -tubes and stored at (2-8 ^{o}C) for HbA_{1c} measurement within one week. The remaining blood sample was centrifuged (at 3000 rpm for 10 minutes) for the determination of fasting glucose and postprandial blood glucose. The remaining serum was frozen at (–20^{o}C) for estimation of serum insulin and omentin-1 levels.

Determination of fasting blood glucose (FBG) levels: Fasting blood glucose level was determined by using a ready-made kit (Biolabo, Glucose GOD-PAP, France) on the KENZA 240 TX Automatic Biochemistry Analyzer, based on method of Borham and Trindoer.

Determination of serum insulin levels: Serum insulin level was determined using a ready-made kit (accubind, ELISA Microwells, Monobind Inc., USA), the insulin ELISA kit is a solid phase ELISA depending on the sandwich principle. The absorbance is spectrophotometrically measured at 450 nm.

Determination of Insulin Resistance (IR): Insulin resistance was determined from the HOMA-IR (homeostasis model assessment of insulin resistance) depending on the following formula [20]:

$$HOMA - IR = \frac{\text{fasting serum insulin concentration } \left(\frac{\mu U}{ml}\right) \text{x FBG } \left(\frac{mg}{dl}\right)}{405}$$

Determination of HbA_{1c}:HbA_{1c} level was determined using a ready-made kit (Clover A1c, Infopia Inc., Anyang, Korea) based on boronate affinity method [21].

Determining Serum Omentin-1 Levels: Serum omentin-1 concentrations were determined using a commercially available ELISA kit (Biovendor, Czech). All kit measurements were according to the kit constructions.

Analysis of the current data was carried out using of Statistical Packages for Social Sciences- version 22(SPSS-22). Data were presented in simple measures of mean, percentage, frequency, range and standard deviation.The significance of difference of different percentages (qualitative data) was tested by using Pearson Chi-square test (χ^2-test). The significance of difference was tested by using paired-t-test for difference of paired observations regarding p value <0.05 as significance.

RESULT

Sixty patients were included in this study, thirty newly diagnosed T2DM patients started with metformin treatment and thirty healthy volunteers were regarded as control group. Demographic data and baseline characteristics were not significantly different among the two groups, table (1). There was a non-significant difference in the baseline BMI values between metformin group and control group (p=0.476). Within the same group, there was a non-significant difference in BMI values after three months of treatment compared with baseline values and metformin group. There was a significant difference in the baseline fasting blood glucose, postprandial blood glucose and HbA_{1c} levels between metformin and control groups (p=0.0001). Within the same group, there was a significant decrease in these parameters after three months of treatment compared with baseline values in metformin group.

There was a non significant difference in the baseline serum insulin levels between metformin and control groups (p=0.136). Within the same group, there was significant difference in serum insulin levels after three months of treatment compared with baseline values in metformin group p<0.01. There was a significant difference in the baseline insulin resistance values between metformin and control groups (p=0.0001). However, within the same group, there was a non significant difference in insulin resistance values after three months of treatment compared with baseline values and metformin group. There was a significant difference in the baseline serum omentin-1 levels between metformin and control groups (p=0.023); and significant raising in serum omentin-1 levels after three month s duration of treatment with metformin, table (2).

Table 1. Demographic data and baseline characteristics of the patients and control groups.

Variables		Control group (n=30)	Metformin group (n=30)	*P* value
Age (*yr*)		47.80±9.31	49.32±11.17	0.104
Gender (no.)	Male	20	24	0.126
	Female	10	6	
BMI (kg/m^2)		28.52±4.02	29.28±4.18	0.454
Smoker (no.)		7	5	0.519

Data presented as mean ± SD; and numbers.

Table 2. effects of metformin on anthropometric and biochemical measures on type 2 DM before and after three months of treatment.

	Control		Metformin	
Variables	Before (Mean ±SD)	After (Mean ±SD)	Before (Mean ±SD)	After (Mean ±SD)
BMI (Kg/m^2)	28.52±4.02	28.342±3.02	29.28±4.18	28.25±5.56
F BG (mg/dl)	93.60±8.73	90.60±8.73	228.56±57.57¶	206.44±71.64**
PP G level (mg /dl)	116.87±11.22	117.67±18.22	228.56±57.57¶	148.56±11.47**
HbA1C (%)	5.03±0.32	5.03±0.31	10.20±1.76¶	7.74±1.76**
Serum Omentin (mIU/dl)	10.25±7.20	10.15±4.25	6.59±4.84¶	8.84±7.27*
Serum insulin (µIU/ml)	10.46±6.30	10.48±5.30	14.11±9.40¶	10.32±8.10**
Insulin Resistance	2.38±1.41	2.25±1.22	6.40±4.95¶	4.11±3.43*

P*<0.05;p*<0.01(metformin versus control after three months duration); ¶*p*< 0.05 (baseline metformin versus control (before)**

The correlations of serum omentin-1, serum insulin and insulin resistance with other parameters (pre- and post-treatment) in metformin group. With respect to insulin resistance in metformin group, the results showed that insulin resistance value at baseline was positively correlated with fasting blood glucose, HbA_{1C}, and fasting serum insulin. After three months of treatment with metformin, insulin resistance value was significantly positively correlated with fasting blood glucose and fasting insulin levels. Fasting insulin levels were significantly correlated with insulin resistance values, both at baseline and after three months of treatment with metformin.Moreover, in the present study, serum omentin-1 is negatives correlated with serum insulin at baseline status r= -0.3,.After three

months of treatment with metformin, serum omentin-1 was correlated with serum insulin at post-treatment with metformin r= -0.17. At pretreatment stage, serum omentin-1 was correlated with insulin resistance, r=-0.271, but this correlation becomes low after three months of treatment with metformin r=-0.059 . table 3.

Table 3. Correlations of serum omentin-1, serum insulin and insulin resistance in metformin group with other parameters pre- and post-treatment.

Parameters		Omentin 1 (MIU/dl)		IR level		Fasting Plasma Insulin (MIU/dl)	
		Before	After	Before	After	Before	After
Fasting blood glucose (mg/dl) Before	r	-0.129	0.102	0.444*	0.562**	0.017	0.569**
	P	0.491	0.586	0.012	0.001	0.927	0.001
Fasting blood glucose (mg/dl) After	r	-0.055	0.034	0.516**	0.547**	0.335	0.236
	P	0.768	0.855	0.003	0.001	0.065	0.201
HbA_{1C} (%) Before	r	-0.222	0.226	0.486**	0.490**	0.223	0.404*
	P	0.229	0.221	0.006	0.005	0.229	0.024
HbA_{1C} (%) After	r	-0.041	0.054	0.424*	0.294	0.293	0.136
	P	0.827	0.774	0.018	0.108	0.110	0.465
Omentin- 1 (MIU/dl) Before	r	-	0.132	-0.271	-0.200	-0.300	-0.232
	P		0.480	0.141	0.280	0.100	0.210
Omentin 1 (MIU/dl) After	r	0.132	-	0.211	-0.059	0.122	-0.171
	P	0.480		0.254	0.751	0.512	0.358
Fasting Plasma Insulin (MIU/dl) Before	r	-0.300	0.122	0.874**	0.056	-	-0.047
	P	0.100	0.512	0.0001	0.763		0.800
Fasting Plasma Insulin (MIU/dl) After	r	-0.232	-0.171	0.163	0.924**	-0.047	-
	P	0.210	0.358	0.382	0.0001	0.800	
Post prandial glucose level (mg/dl) Before	r	0.039	0.074	0.077	0.044	-0.121	0.107
	P	0.835	0.692	0.679	0.812	0.516	0.567
Post prandial glucose level (mg/dl) After	r	-0.049	0.030	0.234	0.252	0.180	0.206
	P	0.794	0.873	0.206	0.171	0.331	0.267
IR level Before	r	-0.271	0.211	-	0.290	0.874**	0.163
	P	0.141	0.254		0.113	0.0001	0.382
IR level After	r	-0.200	-0.059	0.290	-	0.056	0.924**
	P	0.280	0.751	0.113		0.763	0.0001
BMI (Kg/m^2) Before	r	0.136	0.142	-0.031	0.182	-0.101	0.184
	P	0.465	0.444	0.869	0.327	0.590	0.322
BMI (Kg/m^2) After	r	0.202	0.108	0.046	0.207	-0.027	0.210
	P	0.276	0.563	0.805	0.263	0.887	0.258

***P<0.05**
****p<0.01**

DISCUSSION

Abdominal fat was found to be a more pathogenic compared to subcutaneous fat in producing IR, T2DM, and cardiovascular events. Adipose tissue has been secretes different active molecules, called adipokines that affect glucose and lipid metabolism. These adipokines include visfatin, resistin, adiponectin, interleukin-6, leptin and TNF-α. Omentin-1 was founded to be preferentially expressed in visceral fat more than subcutaneous fat. Omentin-1 may be a novel hormone act as a modulator of systemic metabolism, including insulin action in subcutaneous adipose tissue [22]. Interestingly, individuals with impaired glucose homeostasis, as well as T2DM, have a reduced serum omentin-1 levels.

Concerning the effects of three months therapy with metformin alone on serum glycemic indices (FBG, PBG, HbA_{1c}, serum insulin and IR), significant reduction was reported in FBG, PBG and HbA_{1c} levels.

The mean value of BMI fora newly diagnosed T2DM was (29.28±4.18 kg/m^2) which occurs at near the upper limit of overweight classification 25–29.9 kg/m^2) [23]. There is a close links between the pathophysiology of obesity and T2DM. Previous studies showed that risk of T2DM was increased directly with body weight and obesity, especially the central obesity which increased

the risk of T2DM by 10-11 folds [24]. A non-significant difference was showed in BMI values after three months of treatment compared with baseline values in metformin group which was agreed that metformin usually produce weight loss and result of the current study was inconsistent with observations done by several authors where significant decreased in body weight was associated with metformin treatment [25].

Metformin therapy decreased fasting serum glucose concentrations significantly that are matched with other studies [26]. The reduction in FBG reported with metformin is mainly as consequence of reduced hepatic glucose output (mainly by inhibiting gluconeogenesis and to a lesser degree, glycogenolysis) and increased glucose uptake by skeletal muscle as well as by adipocytes [27]. The glucose production by liver was reported to increase at least twofold in T2DM [28]. The exact mechanism by which metformin reduces this production still unclear, but its major site of action appears to be the mitochondria of hepatocytes, producing an inhibition of cellular respiration leading to decrease gluconeogenesis [29].

In addition, metformin reduces FFA oxidation by 10-30% [30]. Elevated FFA levels are commonly found in diabetes contribute to increased hepatic glucose output and insulin resistance [31].

Metformin also enhances insulin-induced inhibition of gluconeogenesis from several substances, including glycerol, lactate, pyruvate, and amino acids, with antagonizes glucagon-induced gluconeogenesis [32].

Postprandial blood glucose is reduced by metformin therapy either by increasing splanchnic utilization of glucose or through enhancing the peripheral uptake of glucose since; at gastrointestinal tract, there is no significant effect of metformin on glucose absorption [33]. However, metformin was found to accumulate in the gastrointestinal wall that favors glucose metabolism to for lactate [34]. The combination of these effects will lowers postprandial glucose level. Moreover, metformin increases uptake of glucose by the muscle under conditions of elevated glucose level [35]. In addition, metformin lower plasma glucagon concentrations and antagonize glucagon actions [36]. Finally, an increased post-meal hepatic blood flow by metformin may increases hepatic glucose uptake [37].

The finding of increased insulin resistance in newly diagnosed T2DM enrolled in the current study was in agreement with a well-established finding that insulin resistance is a characteristic pathological abnormality of T2DM and considered an essential contributor for the development of T2DM [38]. However, significant reductions were revealed in insulin levels and insulin resistance after three months of metformin treatment, findings that were compatible with findings of other studies in which metformin, decreases insulin resistance in T2DM patients, with subsequent decrease in baseline as well as glucose-stimulated insulin secretions [39]. Concerning the newly diagnosed T2DM patients who started treatment with metformin, the results showed that newly diagnosed T2DM patients had significantly lower serum omentin-1level. Omentin-1 is relatively a newly identified adipokine and previous studies showed negative correlations between omentin-1 serum levels and gene expression with T2DM [40]. In vitro study had shown that omentin-1 increases glucose uptake by human adipocytes through enhancing protein kinase B (AKT) phosphorylation and transduction of insulin signal [41]. The findings of the current study confirm that T2DM patients demonstrated decreased levels of omentin-1. The reduced levels of omentin-1 reported in T2DM patients may lead to a reduction in insulin-mediated uptake of glucose in both visceral and subcutaneous fats as well as other insulin-sensitive tissue [42]. However, it may be difficult to determine whether a high glucose level is the cause or result of low serum omentin-1 levels and by which mechanisms glucose affects omentin-1 level. Bee *et al.*, found that both glucose as well as insulin significantly decrease the omentin-1 production in adipose tissue explants in a dose-dependent manner and that a high insulin level significantly reduced levels of serum omentin-1in healthy individuals [43]. Thus, omentin-1 synthesis is regulated directly or indirectly by plasma glucose and insulin levels [44]. In addition, other possible factors may contributes for decreasing serum omentin-1 levels in newly diagnosed T2DM which could be excessive body weight since; mean of BMI for a newly diagnosed T2DM involved in the current study was 29.28±4.18 kg/m^2,according to BMI Classification, those patients are considered overweight (BMI between 25–29.9 kg / m^2) [45].

This possibility is matched with finding of increased level of omentin-1 in patients with anorexia nervosa associated with significant reduced body fat stores [149]. It was observed that lean individuals had significantly increased serum omentin-1levels compared to obese or overweight patents [46].

Following three months treatment of a newly diagnosed T2DM with metformin, there was no significant change in the level of omentin-1 compared to baseline level. Serum omentin-1 levels were reported to increases following metformin treatment in women with PCOS , and following treatment of metformin plus liraglutide in T2DM Chinese patients [47]. There was significant effect of metformin therapy on omentin-1 levels in the

current study, which be attributed to the differences among the patients characteristics between the current study the Chinese study such as gender distribution, ethnicity, as well as lack of a 'metformin monotherapy' group in the Chinese study [48].

Conclusions

In a newly diagnosed T2DM patients, omentin-1 levels were lower compared to control subjects. Three months of treatment with metformin lead to significant elevation in omentin-1 serum levels compared with baseline values.

Recommendations for Future study

Further studies are needed to investigate the effects of metformin combined with SGLT2 inhibitors or sitagliptin on serum omentin-1 level.

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